Surface Passivation & Speed Effects of Molecular Motor Protein Assays

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"Kiney"

Open Notebook Science

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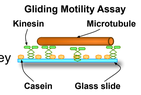
Motivation

Kinesin and microtubules have been proposed to be used as components for chemical and biological sensors. In order to fully understand the dynamics of kinesin and microtubules, fundamental questions must be answered related to how we observe kinesin and microtubule interactions. One way to observe their interactions is by using a ***gliding motility assay*** which can easily be visualized as a molecular "crowd surfing" for microtubules. In order for kinesin to support microtubule crowd surfing, the glass the kinesin is on must be functionalized in order to passivate the surface. Understanding the interactions of kinesin with this passivation is crucial to understanding novel ways to engineer MEMS devices for the detection of chemical and biological materials.

Introduction

Surface passivation is crucial for kinesin and microtubule experiments. Without passivation, ***gliding motility assays*** will fail. The current beliefs as to why failure occur include[1]:

- No structural support for kinesin.
- Kinesin denatures when it hits glass.
- Motor domains may inactivate when they
- contact glass.



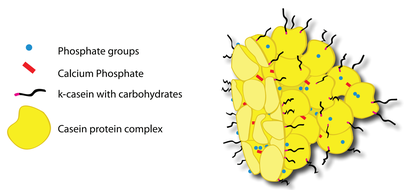
To run experiments, a protein called ***casein*** is used to passivate the glass. There are many issues with using casein, some of them include:

- It is designed to carry calcium phosphate to infant mammals. This calcium could aid in depolymerizing microtubules
- No crystal structure exists.
- Its use is based on legacy.

Casein[2]

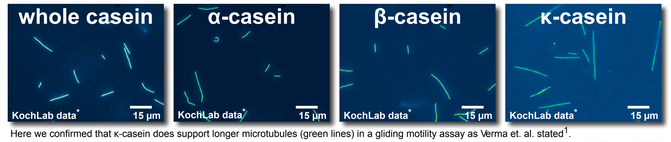
- Globular protein.
- Makes up 80% of the protein content in bovine milk.
- Consists of 4 major subunits, α_{s1}, α_{s2}, β, κ.
- Carries calcium phosphate to infant mammals.
- Amphiphilic.

An issue that makes casein unattractive as a surface passivator is that it is designed to deliver calcium to infant mammals. Calcium is a known depolymerizing agent for microtubules. Its use is also based on legacy which has not been fully investigated. To fully elucidate the physics behind kinesin and microtubule interactions a simpler, cleaner surface passivator must be found. Knowing the physics behind the interactions will greatly expedite the design of novel sensors.

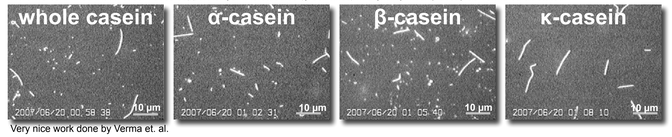


Surface passivation with casein

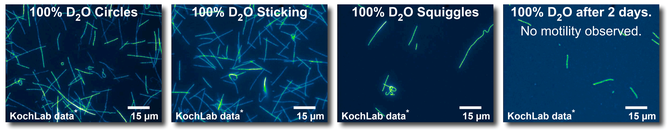
- To the best of our knowledge, only casein and Bovine Serum Albumin (BSA)[3] have been successfully used as surface passivators for gliding motility assays.
- Whole casein is notoriously difficult to get into solution. Alpha, beta, and kappa casein will go into solution with gentle, constant stirring.
- This is a classic case of legacy usage. Casein proteins do not denature when exposed to excess heat[4]. However, scientists have devised very clever ways of getting whole casein into solution without heating.
- Different components of casein support microtubules differently[1]. Our initial findings suggest that all the constituents of bovine casein can be used to passivate glass for successful motility assays.
- There is no consensus in the scientific community as to why casein supports gliding motility assays. It could be that casein props up kinesin or it could be that casein binds to kinesin's tail or stalk region thus allowing it to keep its motor domains free so that they can interact with the microtubules in solution.



Here we confirmed that κ-casein does support longer microtubules (green lines) in a gliding motility assay as Verma et. al. stated[1].



Very nice work done by Verma et. al.

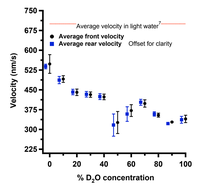


Microtubules, Kinesin & D_2O

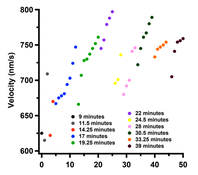
- The kinetics of kinesin and microtubules in heavy water has not been studied up till now.
- Some interesting effects occur when kinesin and microtubules are added to a heavy water motility assay including: circles, sticking, and squiggles.
- Another interesting fact is that microtubules are stabilized in heavy water as is evident in their persistence after 2 days. Studies[5,6] have shown that tubulin is more stable in heavy water.
- Fluorescence is also enhanced. Microtubules remain fluorescent for much longer times. Studies[9] have shown that rhodamine used in dye lasers can benefit from being in a solution of D_2O.

Gliding Motility Assay Speeds

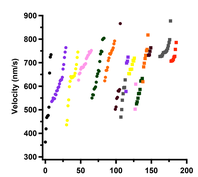
- The speed at which kinesin is able to move crowd surfing microtubules is dependent on many factors. Some known speed affectors include divalent ion concentration such as Mg^{2+} or Ca^{2+} in solution, ATP concentration, kinesin density on the slide, and viscosity of the buffer solution.
- Heavy water also affects the speed, and the effect is dependent on heavy water concentration. Original D_2O data taken with an early version of tracking software.
- Time is also an unusual factor. Most studies prepare slides and wait >15 minutes before observing, however, our data show that there is an affect caused by a yet unknown factor that causes the speed of microtubules to increase over extended observation times.



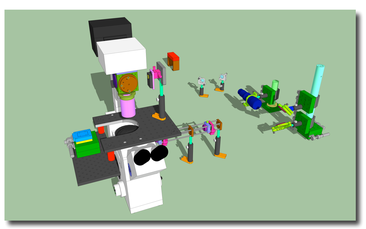
Heavy water speed variations.



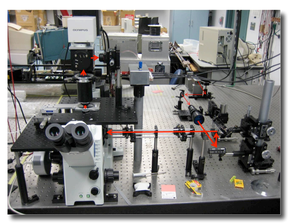
Time based speed variations. β-casein slide pasivation.



Time based speed variations. Whole-casein slide pasivation.



3D rendering of the KochLab optical tweezers. Individual optical components were drawn in Google Sketchup by Maloney except for the microscope which was done by ***jpkleman***. All models are freely available through Google's 3D warehouse.



KochLab optical tweezers. Working setup for KochLab's optical tweezers. Its primary use is for Shotgun DNA mapping. More information on Shotgun DNA mapping can be found at poster number **B788** on Monday.

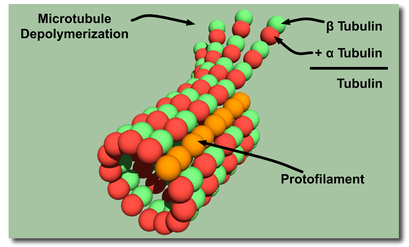
Future work

- ***Osmotic stressors can affect microtubule crowd surfing speeds.*** Preliminary results using sucrose (data not shown) show that the osmotic pressure of a sample can affect assay speeds. Using other small noninteracting molecules such as Betaine may have interesting effects on speed as well. Betaine is also not as viscous as sugar molecules so the viscosity of the solvent will not play a major role in speed variations.
- ***Heavy water solvent.*** Heavy water (D_2O) has been shown that it affects speeds. However, the speeds observed using D_2O as the solvent may be from kinetic isotope effects. Using heavy oxygen water ($H_2{}^{18}O$) will not have the same kinetic isotope affect as D_2O, yet it may show speed variations.
- ***Speed variations with different caseins.*** The major components of bovine caseins used as surface passivators may have different affects on motility speeds. As was shown, the different caseins do affect the sizes of microtubules available for crowd surfing which may in turn lead to speed variations for the assay.
- ***Optical tweezers.*** All experiments thus far have been executed in a light microscope using gliding motility assays. The next step is to use an optical trap (designed and built by Maloney and Salvagno) to investigate kinesin attached to a dielectric bead. The optical trap will track the kinesin's motion along a microtubule thus giving us single molecule resolution of kinesin's processivity. Please see the poster on Monday **B788** for more information about the KochLab optical tweezers.

- For more information about the experiments, please see Maloney's open notebook at: **http://www.openwetware.org/wiki/User:Andy_Maloney**

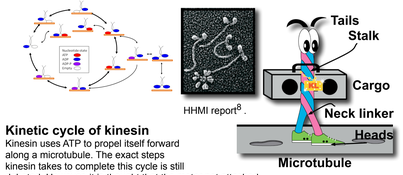
Microtubules

- Heterodimer of tubulin subunits α and β. One α and one β subunit together is called tubulin
- Tubulin forms polymers called protofilaments.
- Microtubules are made from 13 - 17 protofilaments.
- They are hollow and are an average of 25 nm in width.
- Calcium causes depolymerization.



Kinesin

- Dimer that consists of two heavy chains and two light chains.
- The heavy chains form the "head" group or the motor domains.
- The light chains form the "tail" group where cargo binds. Kinesin supplied to us from Dr. Liu does not have light chains. It is a truncated heavy chain Drosophila kinesin-1.
- The chains are connected by a "neck linker" and an intertwined stalk region.
- Uses ATP to generate motion.
- Please see the poster on Monday **B719** for more information on kinesin's kinetic cycle.



Kinetic cycle of kinesin
Kinesin uses ATP to propel itself forward along a microtubule. The exact steps kinesin takes to complete this cycle is still debated. However, it is thought that the motor not attached to the microtubule will not rebind to it, to take a step, until an ATP binds to the motor attached to the microtubule. Binding and unbinding of kinesin motor domains to microtubules will be affected by osmotic stress and isotope water substitutions. These are experimental knobs we wish to turn in order to understand more fundamentally the kinesin catalytic cycle.

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10. CINT is a user facility. More information can be found here, http://cint.lanl.gov/

* Images false colored in ImageJ.